WHAT IS CLAIMED IS:

1. A method for extending a primer or a pair of primers using an enzymatic cycle primer extension reaction at temperatures below about 80°C, comprising the step of mixing a template DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture thereof, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C and an annealing temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers.

2. The method of claim 1, wherein the glycerol, ethylene glycol, or mixture thereof is present in about 15% (v/v).

3. The method of claim 1, wherein the DNA polymerase has an optimum enzymatic activity at about 65°C.

4. The method of claim 1, wherein the DNA polymerase has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus.*

5. The method of claim 1, which comprises the further step of repeating the cycle primer extension reaction.

6. The method of claim 1, wherein copies of a selected segment of a double-stranded DNA are amplified in the presence of a forward primer and a reverse primer to the template by repeated heating and cooling cycles.

7. The method of claim 6, wherein the forward primer and reverse primer may be of varying lengths.

8. The method of claim 1, wherein molecules of a single primer of various lengths are extended with specific nucleotide terminations in the presence of ddNTPs or their analogs for cycle sequencing.

9. A method for extending the molecules of a primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, comprising the steps of:

(i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or mixture thereof, and

(ii) effecting cycle primer extension reaction(s) at a temperature below 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs.

10. The method of claim 9, wherein in vitro amplified double-stranded DNA products are generated by extending a primer or a pair of primers using a enzymatic cycle primer extension reaction at temperatures below about 80°C, comprising the step of mixing a target segment of DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* oand *Bacillus caldolyticus*, in a solution containing about 10% to about 20% (v/v) glycerol, ethylene glycol, or a mixture thereof, under conditions that the

cycle reaction temperature fluctuates between a melting temperature of about 70°C and a cooling temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers.

11. The method of claim 9, wherein the moderately thermostable DNA polymerase has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus.*

12. A dry or liquid ready-to-use reaction mixture suitable for use in a low-temperature cycle primer extension reaction at temperatures below about 80°C, comprising a moderately thermostable, natural or modified DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*, that is pre-mixed with at least one enzymatic DNA primer extension reaction component suitable for use in DNA amplification or for specific extension terminations with dideoxyribonucleotide analogs.

13. The ready-to-use reaction mixture of claim 12. wherein the moderately thermostable DNA polymerase is a natural or modified DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*, or a moderately thermostable DNA polymerase which has an amino acid sequence that shares not less than 95% homology with a DNA polymerase isolated from *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus.*

14. The ready-to-use reaction mixture in claim 12, which is pre-distributed into microcentrifuge tubes or in multiple-well plates.

15. The ready-to-use reaction mixture in claim 13, which is pre-distributed into microcentrifuge tubes or in multiple-well plates.

16. The ready-to-use reaction mixture of claim 14, which is pre-distributed into microcentrifuge tubes or in multiple-well plates, and remains stable at temperatures between 22°C and 25°C for at least eight weeks.

17. The ready-to-use reaction mixture of claim 15, which is pre-distributed into microcentrifuge tubes or in multiple-well plates, and remains stable at temperatures between 22°C and 25°C for at least eight weeks